

CLAIMS

1. Method of obtaining dendritic cells, characterized in that it consists in:

5 1) cultivating, for 4 to 6 days, mononuclear cells derived from cytapheresis after mobilization, in a serum-free medium supplemented with human albumin, in the presence of a granulocyte-macrophage colony stimulating factor (GM-CSF) and an interleukin (IL) that blocks differentiation towards the macrophagic pathway;

10 2) adding TNF- α and optionally an inflammatory mediator to the culture medium and continuing the culture for about a further 1 to 4 days; and

3) recovering the dendritic cells formed.

15 2. Method according to claim 1, characterized in that the culture of step 1) is carried out for 5 days and that of step 2) for 2 days.

3. Method according to claim 1 or 2, characterized in that the interleukin is interleukin-4 or interleukin-13.

20 4. Method according to any one of claim 1 to 3, characterized in that the inflammatory mediator is tumor necrosis factor alpha (TNF- α).

5. Method according to any one of claims 1 to 3, characterized in that the inflammatory mediator is tumor necrosis factor alpha (TNF- α) and prostaglandin E2 (PGE2).

25 6. Method according to any one of claims 1 to 5, characterized in that the mononuclear cells are obtained by cytapheresis after mobilization by chemotherapy and/or with at least one cell growth factor.

7. Method according to any one of claims 1 to 6, characterized in that GM-CSF, interleukin and TNF- α are each used at a rate of 1 to 1000 ng/ml of medium.

30 8. Method according to any one of claims 1 to 7, characterized in that human albumin is used at a rate of 1 to 2% (weight/volume of medium).

9. Method according to any one of claims 1 to 8, characterized in that human albumin is used at a rate of 2% (weight/volume of medium).

35 10. Irreversible dendritic cells, characterized in that they are $\alpha\beta3^-$, $\alpha\beta5^+$, CCR5 $^-$ and CCR7 $^+$.

11. Use of $\alpha\beta3^-$, $\alpha\beta5^+$, CCR5 $^-$ and CCR7 $^+$ irreversible dendritic cells for the preparation of an immunotherapeutic agent useful for the treatment of any disease involving the immune system.

12. Method of immunotherapy treatment, characterized in that it consists in:

- 1) taking mononuclear cells from a patient to be treated by cytapheresis after mobilization by chemotherapy and/or with a cell growth factor and optionally freezing/thawing;
- 2) cultivating, for 4 to 6 days, mononuclear cells derived from cytapheresis after mobilization, in a serum-free medium supplemented with human albumin, in the presence of a granulocyte-macrophage colony stimulating factor (GM-CSF) and an interleukin (IL) that blocks differentiation towards the macrophagic pathway;
- 3) adding TNF- α and optionally an inflammatory mediator to the culture medium and continuing the culture for about a further 1 to 4 days while activating them with specific antigens;
- 4) recovering the dendritic cells formed and activated in this way; and
- 5) reinjecting said dendritic cells into said patient.

13. Method according to claim 12, characterized in that said dendritic cells are frozen/thawed before being reinjected into said patient.

Adattu